

Population Pharmacokinetics of Fluconazole in Young Infants[∇]

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Fluconazole is being increasingly used to prevent and treat invasive candidiasis in neonates, yet dosing is largely empirical due to the lack of adequate pharmacokinetic (PK) data. We performed a multicenter population PK study of fluconazole in 23- to 40-week-gestation infants less than 120 days of age. We developed a population PK model using nonlinear mixed effect modeling (NONMEM) with the NONMEM algorithm. Covariate effects were predefined and evaluated based on estimation precision and clinical significance. We studied fluconazole PK in 55 infants who at enrollment had a median (range) weight of 1.02 (0.440 to 7.125) kg, a gestational age at birth (BGA) of 26 (23 to 40) weeks, and a postnatal age (PNA) of 2.3 (0.14 to 12.6) weeks. The final data set contained 357 samples; 217/357 (61%) were collected prospectively at prespecified time intervals, and 140/357 (39%) were scavenged from discarded clinical specimens. Fluconazole population PK was best described by a one-compartment model with covariates normalized to median values. The population mean clearance (CL) can be derived for this population by the equation $CL \text{ (liter/h)} = 0.015 \cdot (\text{weight}/1)^{0.75} \cdot (\text{BGA}/26)^{1.739} \cdot (\text{PNA}/2)^{0.237} \cdot \text{serum creatinine (SCRT)}^{-4.896}$ (when SCRT is >1.0 mg/dl), and using a volume of distribution (*V*) (liter) of $1.024 \cdot (\text{weight}/1)$. The relative standard error around the fixed effects point estimates ranged from 3 to 24%. CL doubles between birth and 28 days of age from 0.008 to 0.016 and from 0.010 to 0.022 liter/kg/h for typical 24- and 32-week-gestation infants, respectively. This population PK model of fluconazole discriminated the impact of BGA, PNA, and creatinine on drug CL. Our data suggest that dosing in young infants will require adjustment for BGA and PNA to achieve targeted systemic drug exposures.

Despite the significant advances made in the care of seriously ill preterm and term newborns over the past decades (15, 24), significant challenges remain. Treatment or prevention of serious or life-threatening infections in this population is often suboptimal. Neonatal candidiasis is one of the more challenging infections to manage as it is frequently associated with long-term morbidity and too often proves fatal (3–7, 16, 22). One potential reason for limitations of current therapy is that the necessary data to make informed decisions about adequate antifungal dosing are lacking. The cornerstone of effective dosing strategies rests on adequately conducted pharmacokinetic (PK)/pharmacodynamic studies, but such studies have usually proved elusive in the neonatal population. As a result, most antifungal medications lack an FDA-approved indication in neonates, and therapy remains suboptimal.

Fluconazole is a potent antifungal azole drug used for both prevention and treatment of candidiasis (10, 14, 17, and 19; Diflucan package insert, Pfizer, New York, NY). Fluconazole administered either orally or intravenously has an excellent

safety profile in adults and children. It is minimally metabolized, is eliminated as active drug in the urine, and effectively penetrates tissues and cerebrospinal fluid. Although it is frequently used in the neonatal population, remarkably little fluconazole PK data have been published for neonates, with studies being limited to a small number of preterm 24- to 29-week-gestation neonates (9, 21, 23). Existing data are thus insufficient for the development of a generalized dosing guidance. We therefore performed a population PK study in young infants who were receiving fluconazole as routine clinical care for prevention or treatment of systemic candidiasis in the neonatal intensive care unit.

Portions of this work were presented at the Society for Pediatric Research Annual Meeting (Honolulu, HI, 2 to 6 May 2008).

MATERIALS AND METHODS

Study design. Fluconazole samples for this analysis were obtained from two studies enrolling concurrently within the Pediatric Pharmacology Research Unit (PPRU). The following inclusion criteria were the same for both studies: infants with a 23- to 42-week gestation who were less than 120 days old and who were receiving intravenous fluconazole as routine care for prevention or treatment of candidiasis. The primary study (study 1) was an open label, fluconazole PK study conducted at eight institutions (see Acknowledgments). Infant enrollment was stratified by gestational age at birth (BGA) (23 to 25 weeks, 26 to 29 weeks, 30 to 33 weeks, and ≥ 34 weeks) and postnatal age (PNA) (<14 days and 14 to 119

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days). The second study (study 2) was an open label PK study of a panel of antimicrobial drugs being conducted at Duke University. For both studies, fluconazole dosing was determined by the routine clinical practice in each unit and no exclusion criteria were used. These studies were approved by the institutional review boards at each respective institution, and informed consent was obtained.

For covariate analysis, we collected the following information: indication for fluconazole, BGA, dates of positive *Candida* cultures, and daily assessments of PNA, postmenstrual age (PMA), weight, urine output (ml/24 h), and respiratory support. Covariates that exhibited time-dependent changes (e.g., weight, age, and creatinine) were permitted to change with time, and the actual value in the data set reflects the observations made at each patient visit. Missing weights were imputed with the last recorded value carried forward for up to 7 days.

Serum creatinine (SCRT) was recorded when obtained for clinical care. Infants are more likely to have SCRT values measured when they have clinical signs of renal insufficiency. Infants with no measured creatinine values were assumed to have a SCRT value of ≤ 1.0 mg/dl. To avoid the impact of missing SCRT data, we created a dichotomized variable, CR, and assigned a value of 0 for time points when the SCRT level was never measured or when it was ≤ 1.0 mg/dl. Likewise, a CR value of 1 was designated for infants with a measured SCRT of > 1.0 mg/dl, and a SCRT value of > 1.0 was imputed for up to 7 days. We used the exponentiated expression $\text{SCRT}^{(\text{CR} \times \theta)}$ to switch between the renal function subtypes.

PK sample collection. Infants in study 1 were randomly assigned to one of two PK sampling schedules (schedule A included preinfusion, end of infusion, and 1, 6 to 8, 24, and 48 h postinfusion; schedule B included preinfusion, end of infusion, and 3, 10 to 12, 24, and 48 h postinfusion). Sampling was initiated as soon as informed consent was obtained, typically corresponding to the first through fifth dose. If an infant remained on fluconazole, trough plasma samples were obtained on days 7, 14, and 21 (± 2 days). Each sample was 300 μ l of blood in EDTA Microtainers. Plasma was separated and removed within 30 min and stored at -20°C . To supplement these PK samples, plasma from residual discarded clinical specimens timed per clinical routine practice (EDTA Microtainers) was collected up to 72 h after the blood was collected. These scavenged plasma samples were frozen at -20°C . Infants in study 2 had samples scavenged from discarded blood in the clinical laboratory. Samples from all sites were shipped on dry ice to Children's Hospital of Philadelphia, where they were stored at -70°C for analysis. To evaluate fluconazole stability in scavenged samples, we determined the stability of fluconazole (0.03 to 9 $\mu\text{g/ml}$) when stored in whole blood in EDTA Microtainers. Fluconazole plasma concentrations were measured after whole blood triplicate samples were stored for 0.5, 24, 48, and 72 h at room temperature.

Liquid chromatography-tandem mass spectrometry assay for detection of fluconazole in neonates. We developed and validated an analytical method for fluconazole detection in human plasma suitable for the small plasma volumes obtainable in neonates. Liquid chromatography-tandem mass spectrometry analysis was conducted on an API 4000 Q TRAP (Sciex, Toronto, Canada) coupled with a Shimadzu high-performance liquid chromatography (HPLC) system (Kyoto, Japan), using an electrospray ionization source in the positive mode and under the following conditions: curtain gas, 19; gas 1 (nebulizer gas), 35; gas 2 (heater gas), 80; CAD gas, medium; TurboIonSpray voltage, 4,250 V; entrance potential, 11 V; collision energy, 25 V; source temperature, 550°C ; and dwell time, 200 ms. The optimized declustering potential and collision cell exit potential were set at 51 and 5 V, respectively. The collision energy was optimized based on the individual fragmentation selected to obtain the most intense precursor to product ion transitions. Fluconazole-D4, with a molecular weight of 311.20, was obtained from SynFine (Ontario, Canada). HPLC separation was performed using a gradient mobile phase of acetonitrile and water in 0.1% formic acid on a Waters XTerra C₁₈ HPLC column (100 by 2.1 mm; 3.5 μm) (Milford, MA) with a Waters XTerra guard column (10 by 2.1 mm; 3.5 μm) (Milford, MA) at a flow rate of 0.2 ml/min. Multiple reaction monitoring was used to detect fluconazole and fluconazole-D4 (internal standard) at 307.20/219.80 and 311.20/222.80 m/z , respectively. Analytical data were acquired by Analyst software (version 1.4.1). The lower limit of quantitation of fluconazole in plasma was 0.01 $\mu\text{g/ml}$. Intraday and interday coefficients of variation are $< 8.1\%$ at concentrations ranging from 0.01 to 10 $\mu\text{g/ml}$.

Population PK analysis. Pharmacokinetic data were analyzed with a nonlinear mixed effect modeling (NONMEM) approach using the computer program NONMEM (version 5) (3). The first-order conditional estimation method was used for all model runs. One- and two-compartment structural models were evaluated. Interindividual random effects were evaluated on clearance (CL) and volume of distribution (V). Covariance was described by a block Omega matrix. We used an exponential model for interindividual variance for CL and V . A combined additive and proportional error model was deemed appropriate to describe residual variability. The potential impact of physiologically plausible,

clinical covariates on PK parameters was explored in a forward stepwise manner as follows: weight (kg), allometric scaling, BGA (weeks), PNA (week of life; defined as day of life/7), PMA (defined as BGA plus PNA in weeks), and SCRT. Covariates were retained in the final model if there was an improved goodness of fit and a decreased minimum objective function. A drop in objective function of > 10.83 , considered significant at $P < 0.001$, was used to discriminate among alternative nested models. Continuous covariates were scaled to their median values. Empirical Bayesian estimates of individual infant PK parameters were generated from the final model using the POSTHOC subroutine.

Model evaluation. Models were evaluated based on the following six criteria: (i) successful minimization, (ii) goodness of fit as assessed by the Akaike information criterion (18), (iii) diagnostic plots, (iv) precision of parameter estimates, (v) an internal quantitative predictive check, and (vi) an external visual predictive check. We assessed precision of the final population PK model parameter estimates using stratified nonparametric bootstrapping (1,000 replicates) to generate the 95% confidence intervals (CIs) for parameter estimates. For the internal predictive check, the quantity of interest was the average observed fluconazole concentration in each infant. Summary metrics of the quantity of interest were calculated for the observed values in this study cohort and compared to values obtained from 1,000 Monte Carlo simulation replicates of the original data set using the final population PK model. For the external predictive check, the final model was used to generate 100 Monte Carlo simulation replicates of fluconazole exposure in the fluconazole PK preterm neonatal cohort described by Saxén et al. (21), and simulated results were compared with those observed in the study. For prediction into the Saxén data set, we excluded SCRT from the model, since only a single SCRT value was available per infant and a different creatinine assay was utilized for babies in the Saxén study (21).

Assessment of dose-exposure relationship. Monte Carlo simulations using the final population PK model were used to explore the impact of PNA and BGA on dose-exposure relationships. For target exposure, we chose an area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) of 800 $\text{mg} \cdot \text{h/liter}$. This AUC is consistent with exposures in adult intensive care units or immunocompromised patients treated with fluconazole at 800 mg/day and ensures that exposure exceeds the pharmacodynamic target of an AUC/MIC value of > 50 for *Candida* species with a MIC of 8 $\mu\text{g/ml}$ at the CLSI sensitivity breakpoint (2, 12, 13, 19). We determined the interval AUC_{24} to be the AUC for each 24-h interval after a given dose calculated within the NONMEM control file and derived from the differential equations defining the model. These simulations performed with NONMEM included infants with demographic characteristics of the study population and infants exposed to 12 mg/kg/day fluconazole for 14 days. We excluded exposure predictions when an infant's SCRT was ≥ 1.3 mg/dl because these infants would have a dose adjustment for renal insufficiency. The dose required to achieve a target steady-state AUC_{24} was calculated by multiplying the individual model-predicted CL ($\text{liter/kg} \cdot \text{h}$) by the target AUC_{24} (800 $\text{mg} \cdot \text{h/liter}$).

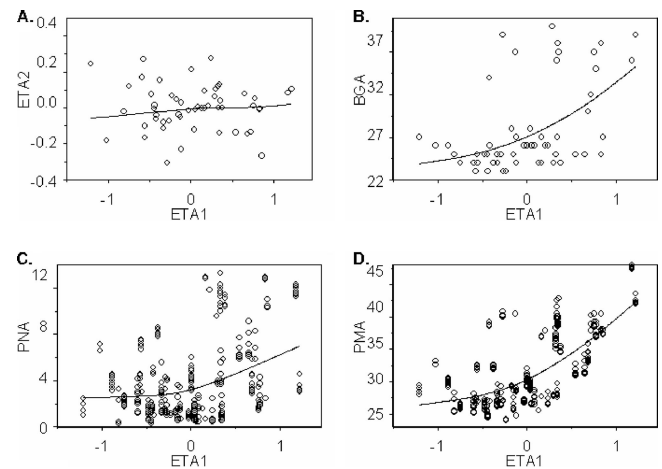
RESULTS

Study infants and PK specimens. Blood samples were collected from 55 preterm and term infants (49 from study 1; 6 from study 2) who were receiving fluconazole as routine care for prevention or treatment of *Candida* infections prior to enrollment (Table 1). Dosing ranged from 3 to 12 mg/kg/dose. We enrolled only three 30- to 33-week-gestation infants who were all more than 14 days old. Term infants enrolled in this protocol typically had multiorgan dysfunction and received fluconazole for fungal sepsis, empirical fungal coverage, or prevention due to severe immunodeficiency. None of the seven infants with *Candida* bloodstream infections had MICs obtained for their *Candida* species. None of the infants receiving fluconazole prophylaxis had breakthrough *Candida* infections.

The final PK data set contained 357 observations: 217/357 (61%) timed samples processed according to the PK protocol and 140/357 (39%) scavenged samples from discarded clinical blood specimens with delayed plasma separation of up to 72 h. An average of 6.5 samples per infant (range, 1 to 16) was collected, with approximately 50% of samples being obtained in 23- to 25-week-gestation infants. Most samples, 247 (60%), were collected during the first month of life,

TABLE 1. Baseline characteristics of infants

Parameter ^a	Value ^b
Infant characteristics	
BGA (wk)	26 (23–40)
PNA (days).....	16 (1–88)
Weight (g)	1,020 (451–7,125)
Gender (% male).....	56
Race or ethnicity (%)	
Caucasian	50
Black	40
Other	10
Hispanic.....	9
No. (%) of infants receiving:	
Prophylaxis from birth.....	23 (42)
Prophylaxis for broad antibiotic exposure.....	11 (20)
Prophylaxis for NEC	8 (15)
Treatment of fungal sepsis	7 (13)
Treatment of fungal UTI.....	2 (3)
Empirical treatment of fungal sepsis.....	4 (7)

^a UTI, urinary tract infection; NEC, necrotizing enterocolitis.^b All values for infant characteristics are the median (range).FIG. 1. Scatter plot of variance term for CL (ETA1) using the base model. Correlation between variances on CL (ETA1) and the following variables: variance on V (ETA2) (A), BGA (B), PNA (C), and PMA (D).

including 68 samples (19%) from the first week of life. Nineteen samples were obtained from the three 30- to 33-week-gestation infants, all after the second week of life.

Population PK model construction. A one-compartment model was the appropriate structural model for this data set based on diagnostic plots (see Fig. 2), and a large coefficient

of variation around estimates of intercompartmental clearance. We began with a base model of CL and V with weight (1) (Table 2). In the base model, scatter plots revealed correlations between interindividual variances on CL (ETA1) and V (ETA2); therefore, a covariance term was added to the model. The residual unexplained variability of

TABLE 2. Construction of population model

Model description ^a	Population model ^d	AIC ^e
Base model and maturity effects ^b		
V	$V = \theta_V \times (\text{wt})^1$	
CL base model		
BGA	$\text{CL} = \theta_{\text{CL}} \times (\text{wt})^{0.75}$	362
PNA	$\text{CL} = \theta_{\text{CL}} \times (\text{wt})^{0.75} \times (\text{BGA})^{\theta_{\text{CL-BGA}}}$	348
PMA	$\text{CL} = \theta_{\text{CL}} \times (\text{wt})^{0.75} \times (\text{PNA})^{\theta_{\text{CL-PNA}}}$	340
	$\text{CL} = \theta_{\text{CL}} \times (\text{wt})^{0.75} \times (\text{PMA})^{\theta_{\text{CL-PMA}}}$	339
Multivariable analysis		
CL BGA and PNA	$\text{CL} = \theta_{\text{CL}} \times (\text{wt})^{0.75} \times (\text{BGA})^{\theta_{\text{CL-BGA}}} \times (\text{PNA})^{\theta_{\text{CL-PNA}}}$	324
CL BGA, PNA, and SCRT if CR is >1 mg/dl	$\text{CL} = \theta_{\text{CL}} \times (\text{wt})^{0.75} \times (\text{BGA})^{\theta_{\text{CL-BGA}}} \times (\text{PNA})^{\theta_{\text{CL-PNA}}} \times \text{SCRT}^{(\theta_{\text{CL-SCRT}})(\text{CR})}$	285
Evaluation of scavenged samples on residual variance		
CL final model ^c	$\text{CL} = \theta_{\text{CL}} \times (\text{wt})^{0.75} \times (\text{BGA})^{\theta_{\text{CL-BGA}}} \times (\text{PNA})^{\theta_{\text{CL-PNA}}} \times \text{SCRT}^{(\theta_{\text{CL-SCRT}})(\text{CR})}$	263
Random error model in which SCAV = 1 if scavenged, otherwise SCAV = 0.		
$Y1 = F \times [1 + \text{ERR}(1)] + \text{ERR}(2)$ for protocol-driven PK specimen.		
$Y2 = F \times \theta_{\text{scav}} \times [1 + \text{ERR}(3)] + \text{ERR}(4)$ for scavenged PK specimens.		
$Y = (Y2 \times \text{SCAV}) + [Y1 \times (1 - \text{SCAV})]$.		

^a All models have an exponential error model to describe interindividual variance on clearance and volume of distribution. Additive and proportional models are used to describe residual variance.^b Covariates standardized to median in cohort.^c Final model with the addition of random error variables to account for scavenged blood samples.^d wt, weight normalized to 1 kg/week (1 week); BGA, normalized to 26 weeks; PNA, normalized to 2 weeks; PMA, defined as BGA plus PNA and normalized to 28 weeks; SCRT, normalized to 1 mg/dl; CR, dichotomous renal function variable equals 1 if SCRT is greater than 1 mg/dl and otherwise equals 0; SCAV, dichotomous variable equals 1 if PK sample is scavenged from discarded blood. Y, composite expression for model-predicted concentrations; Y1, expression for predicted concentration value (normal blood collection); Y2, expression for predicted concentration value (scavenged sample); ERR(1), proportional residual error (normal blood collection); ERR(2), additive residual error (normal blood collection); ERR(3), proportional residual error (scavenged sample); ERR(4), additive residual error (scavenged sample).^e AIC, Akaike information criterion (18).

TABLE 3. Final model population pharmacokinetic parameter estimates

Parameter	Symbol ^a	Point estimate	% RSE ^b	Bootstrap CI ^c		
				2.5%	Median	97.5%
CL (liter/h)	θ_{CL}	0.015	5.9	0.013	0.015	0.017
V (liter)	θ_V	1.024	3.8	0.944	1.021	1.096
CL ~ BGA	$\theta_{CL} - BGA$	1.739	18	1.068	1.768	2.328
CL ~ PNA	$\theta_{CL} - PNA$	0.237	24	0.081	0.232	0.352
CL ~ SCRT; CR = 1 if SCRT > 1 mg/dl, CR = 0 if SCRT ≤ 1 mg/dl	$\theta_{CL} - SCRT(CR)$	-4.896	-22	-9.418	-5.033	-2.721
SCAV error model	θ_{SCAV}	0.953	3.5	0.890	0.954	1.020
Interindividual variance						
Omega 1,1	ω_{CL}^2	0.11	20	0.064	0.104	0.156
Omega 1,2	$\omega_{CL}^2 - V$	0.014	152	-0.033	0.010	0.055
Omega 2,2	ω_V^2	0.057	31	0.025	0.055	0.095
Residual variance						
Sigma 1 nonscavenged PK sample	σ_{prop}^2	0.027	31	0.004	0.025	0.041
Sigma 2 nonscavenged PK sample	σ_{add}^2	0.04	102	0.004	0.042	0.300
Sigma 3 scavenged PK sample	σ_{prop}^2	0.081	34	0.021	0.076	0.128
Sigma 4 scavenged PK sample	σ_{add}^2	0.023	143	0.0000	0.021	0.120

^a prop, proportional; add, additive.^b RSE, percent relative to the standard error of the estimate equals the standard error divided by the parameter estimate times 100.^c 95% CI lower and upper bounds and median as determined from a parametric bootstrap of 1,000 replicates.

CL (ETA1) showed a correlation with indices of age and maturity (Fig. 1). Steps in subsequent model building are shown in Table 2. CL estimated by the model with BGA and PNA together was superior to CL estimated with PMA. No additional maturity affects were added to the model for V since no additional decline in partitioning of variance was seen.

Creatinine CL is difficult to measure in infants and was not available for this cohort. We relied on standard-of-care measurements of creatinine for our best estimate of renal CL. Most (87%) infants had at least one creatinine measurement during PK sampling. However, creatinine measurements were rarely obtained during the first 3 days of life. To avoid the impact of missing data, we added SCRT into the model only if the creatinine was >1.0 mg/dl (CR = 1). Therefore, in the absence of a creatinine measurement during the first 3 days of life, PNA and BGA variables account for changes in renal CL shortly after birth.

We evaluated the impact of scavenged specimen acquisition with delayed separation of plasma from whole blood. Observed fluconazole concentrations from scavenged samples were indistinguishable from concentrations in PK samples on plots of observed versus predicted concentrations. We also confirmed fluconazole stability in whole blood stored at room temperature in the laboratory (data not shown). In the random effects error model, we incorporated a term (SCAV) to be equal to 1 for samples collected from scavenged discarded blood (Table 2). Scavenging introduced minimal bias and appeared to slightly underestimate fluconazole concentrations by 4% (95% CI, -11% to 2%) (Table 3).

Population PK model evaluation. The final model had good precision. Final model parameter estimates are presented in Table 3 with 95% CIs generated by bootstrapping ($n = 1,000$ simulated trials). The percent relative standard error around the parameter point estimates ranged from 3.8 to 24%. Larger relative standard errors were observed for the

point estimates for the additive error model. Goodness-of-fit diagnostic plots are shown in Fig. 2. The internal predictive check of the observed versus model-predicted average fluconazole concentration showed good precision and minimal bias. The external predictive check (Fig. 3) revealed a good fit between observed fluconazole concentrations in the Saxén data set (21) and model-predicted fluconazole concentrations.

Bayesian estimate of CL and V . Given empirical Bayesian estimates of individual PK parameters, we summarized results across the various BGAs and PNAs typical for fluconazole treatment. Based on this analysis, the following three typical infant demographics were considered for examination over the first month of life: a 600-g, 24-week-gestation infant, a 1,000-g, 28-week-gestation infant, and a 1,500-g, 32-week-gestation infant. CL is low at birth: 8, 9, and 10 ml/kg/h for these typical 24-, 28-, and 32-week-gestation infants, respectively. CL doubles in the first month of life to 16, 19, and 22 ml/kg/h for these typical 24-, 28-, and 32-week-gestation infants, respectively. Infants with creatinine levels of greater than 1.3 mg/dl had at least a 70% reduction in fluconazole CL.

Dose-exposure relationship. Monte Carlo simulation was used to evaluate the impact of prematurity, weight, and PNA on the dose-exposure relationship (Fig. 4). We predicted the concentration of fluconazole achieved after a 12-mg/kg daily dose regimen in infants with the age, weight, and prematurity represented in this cohort. For these simulations, we excluded exposure estimates when creatinine was ≥ 1.3 mg/dl since these infants would require a dose adjustment for renal insufficiency. Steady-state AUC is not achieved during the first week (Fig. 4A and B). The average half-life is 30 and 50 h for 23- to 29-week- and 30- to 40-week-gestation infants, respectively. Infants who were less than 30 weeks of gestation achieved a median AUC closer to the target AUC of 800 mg · h/liter (Fig. 4A); infants who were more than 30 weeks of gestation

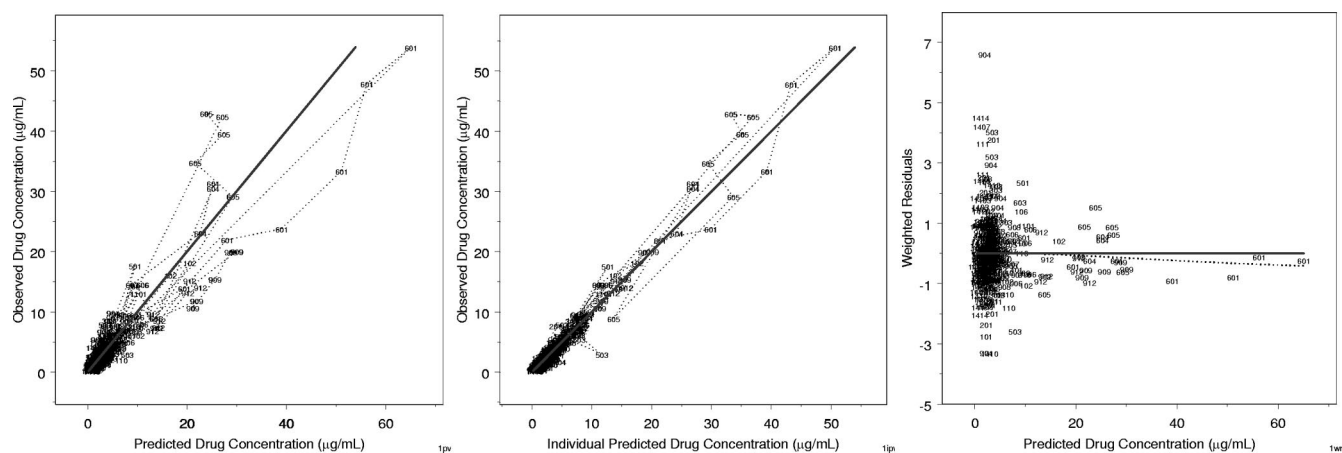


FIG. 2. Final model diagnostic plots of observed versus predicted fluconazole concentration and weighted residuals. For population prediction (A) and individual prediction (B), the individual data points are labeled by individual ID numbers with a dotted line connecting individual data points. Line of identity is included as a reference. For weighted residuals (C), individual data points are labeled by individual ID numbers with a dotted line as a smoothing spline trend line through the data. A solid line at $y = 0$ is included as a reference.

achieved a lower median AUC of $400 \text{ mg} \cdot \text{h/day}$ (Fig. 4B). In order to achieve the $800 \text{ mg} \cdot \text{h/liter}$ AUC target, dosing will need to be adjusted for BGA and PNA (Fig. 4C).

DISCUSSION

Most drugs used to treat neonates remain off-label because of the lack of adequately performed PK studies needed to guide dosing. Limitations in blood volume, limited access for obtaining blood samples, reluctance of families to give consent,

and potential risks associated with dose-finding studies make performance of PK studies in neonates challenging. Novel approaches are needed to enhance PK knowledge of drugs frequently used in neonates. This protocol, designed with these aforementioned constraints in mind, leveraged routine clinical care as much as possible, and thereby proved both efficient and infant friendly.

We developed a population PK model for fluconazole disposition in preterm and term infants less than 90 days of age

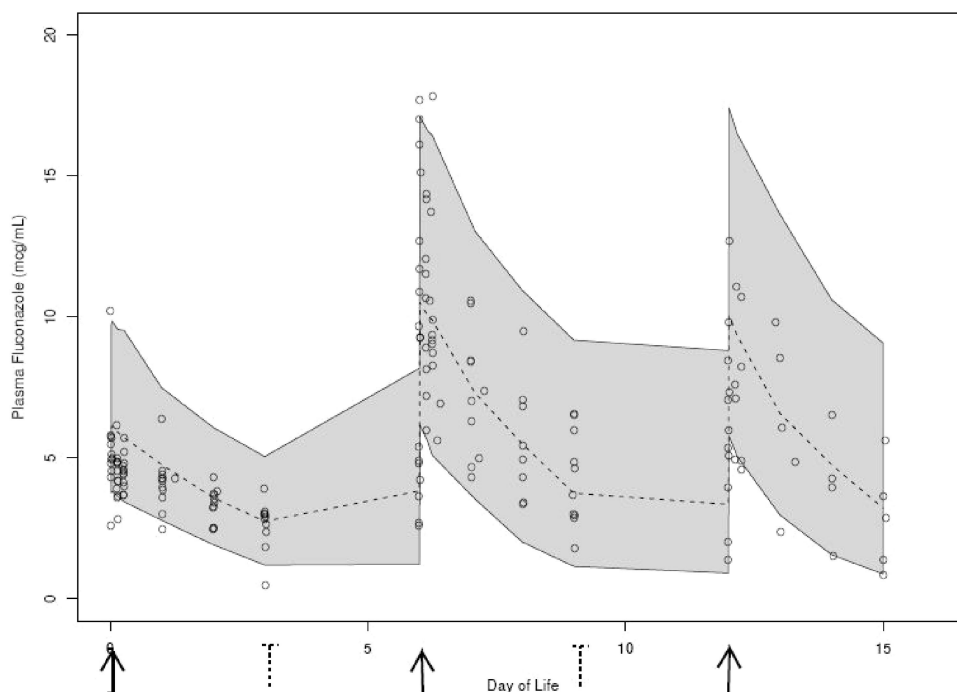


FIG. 3. Predictive check for plasma fluconazole concentration by day of life. Observed fluconazole concentrations (open circles) in a previously published Saxén trial (21) of twelve 26- to 29-week-gestation infants receiving 6 mg/kg fluconazole on day of life 0 (birthday), 3, 6, 9, and 12 are superimposed with the median (dotted line) and 90% population prediction interval (shaded area) for fluconazole concentrations from 100 Monte Carlo simulated trials, given the final model and parameters. Bold arrows indicate fluconazole doses that preceded plasma PK samples. Open lines indicate fluconazole doses that were not followed by plasma PK samples.

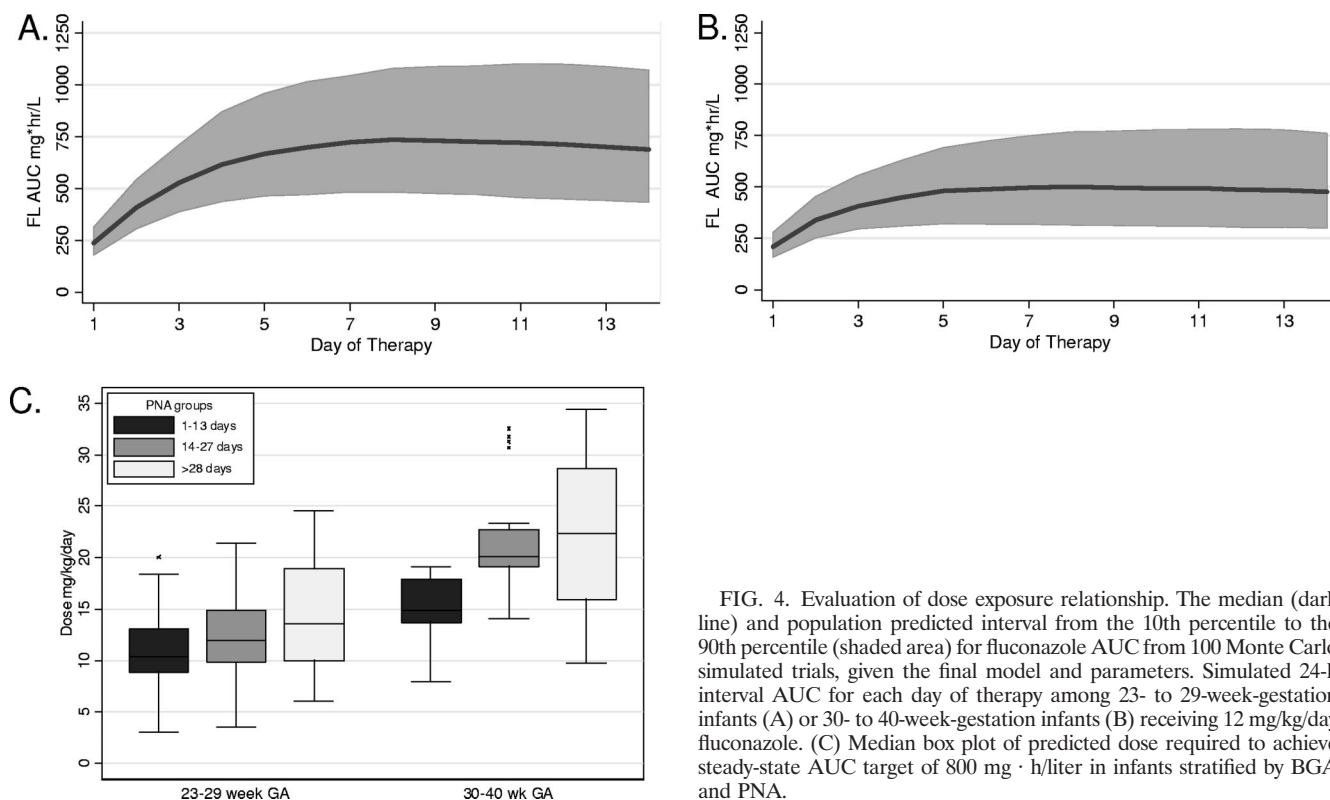


FIG. 4. Evaluation of dose exposure relationship. The median (dark line) and population predicted interval from the 10th percentile to the 90th percentile (shaded area) for fluconazole AUC from 100 Monte Carlo simulated trials, given the final model and parameters. Simulated 24-h interval AUC for each day of therapy among 23- to 29-week-gestation infants (A) or 30- to 40-week-gestation infants (B) receiving 12 mg/kg/day fluconazole. (C) Median box plot of predicted dose required to achieve steady-state AUC target of 800 mg · h/liter in infants stratified by BGA and PNA.

who were receiving fluconazole as part of clinically indicated treatment. Population PK analysis allowed the use of limited sampling schemes and the varied dosing regimens used in routine care. Stratified enrollment ensured broad distribution of both BGAs and PNAs. Randomization to two sampling time schedules allowed for more dense sampling times. Because fluconazole is a stable drug, we were able to increase the number of samples available for analysis by scavenging discarded plasma from clinical specimens. Our robust, sensitive quantitative assay allowed us to accurately measure fluconazole in microvolumes of plasma.

In this model, fluconazole CL increased with allometrically scaled weight (1), BGA, and PNA. PMA, however, did not perform as well as the combination of BGA and PNA. This is not surprising because PMA cannot distinguish a 3-week-old, 24-week-gestation infant from a 1-day-old, 27-week-gestation infant. The model was rigorously evaluated, showed good precision and minimal bias, and was able to estimate into a previously described PK data set in preterm infants (21).

Our model suggests that dose adjustment will be needed to account for the significant changes in fluconazole CL that occurs as a function of BGA and PNA. Although pharmacodynamic efficacy exposure targets for infants have not been described, critically ill or immunocompromised adults with invasive candidemia are typically treated with 800 mg/day of fluconazole to target a steady-state AUC of 800 mg · h/liter. This is double the standard dose required to achieve a pharmacodynamic target AUC_{24}/MIC_{24} of >50 for *Candida*, with the CLSI sensitivity breakpoint MIC being ≤ 8 (2, 12, 13, 19). The higher plasma exposure is a reasonable target to ensure

adequate tissue exposure in patients with widespread, invasive disease (11, 19, 20). Plasma concentrations may not always reveal tissue concentration, and tissue concentrations were not available.

For preterm infants with invasive candidiasis, we chose this target AUC of 800 mg · h/liter because these infants have immature immune systems, have a high projected mortality rate, and often have disseminated disease, including meningoencephalitis (7). A loading dose would be necessary to reach the desired steady-state concentration rapidly. Our data suggest that the lower fluconazole dosages frequently used in the neonatal population result in underexposure and may partially explain the prolonged periods of candidemia (5) and episodes of breakthrough candidemia (23).

Although the neonatologists caring for these infants reported no adverse events, and none of the infants developed a breakthrough fungal infection, a limitation of this study is that comprehensive efficacy and safety data (e.g., liver function tests results, electrocardiograms, etc.) were not collected and thus could not be linked to exposure. Furthermore, we were unable to evaluate drug-drug interactions.

Longitudinal prediction of CL changes with PNA beyond 14 days is difficult. Infants who enrolled when they were more than 14 days old were often receiving fluconazole during periods of clinical deterioration secondary to sepsis or necrotizing enterocolitis. This model may underpredict CL beyond 14 days of age in infants who are relatively well and receiving fluconazole prophylaxis from birth. We plan to perform sparse PK sampling in infants who will be enrolled into a multicenter trial

of fluconazole prophylaxis to improve our longitudinal prediction of changes in CL with PNA in these relatively well infants.

Conclusions. Pharmacokinetic neonatal study designs that leverage routine clinical care can provide reliable PK information. The fluconazole population PK model from this nontraditional design was able to accurately predict the observed fluconazole concentrations in a historic, more traditional PK study. Incorporating both BGA and PNA improved the model fit beyond that achieved with PMA. BGA and PNA effects on CL are significant, and dose adjustments will likely be needed. Our future studies will use Monte Carlo simulation to fully explore dose-exposure relationships, evaluate the effects of a loading dose, and guide further development of pharmacologically rational neonatal dosing recommendations.

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